

Yeast Transformation (introducing plasmid vector into a yeast strain):

This protocol is a modification (shortened version) of “The BEST METHOD” from the Gietz lab “Trafo” website:

(<http://www.umanitoba.ca/medicine/biochem/gietz/Trafo.html>)

1. Inoculate 5 ml of YPD with a yeast colony from plate.
2. Grow culture overnight at 30⁰C.
3. The next day, check the OD600 of the culture and use these cells to start a second 25 ml YPD culture at an OD600 of 0.25.
4. Allow 25 ml culture to grow at 30⁰C for approximately 4 hours.
5. Following growth, culture OD600 should be approximately 1. Harvest cells by transferring to sterile 50 ml conical tube and centrifuging at 1750xg (high speed in clinical centrifuge) for 2 minutes.
6. Carefully pour media off of yeast pellet and resuspend the pellet in 1 ml of sterile nano-pure H₂O.
7. Centrifuge cells again in clinical centrifuge for 2 minutes.
8. Remove the supernatant from the pellet carefully with a pipet.
9. Resuspend the cell pellet in 1 ml of 100 mM lithium acetate and incubate for 5 minutes at 30⁰C.
10. Swirl tube to make sure the cells are suspended, transfer 100 microliters of cells into a 1.5 ml sterile eppendorf tube. Repeat – setting up one eppendorf tube (with 100 microliters of cells) for each transformation reaction.

11. Centrifuge the eppendorf tubes at top speed in a microcentrifuge for 5 seconds (i.e. start microcentrifuge and wait until it *sounds* like it has reached top speed. Count 5 seconds, then stop spin.)
12. Remove the supernatant with a micropipet. Do not disturb the cell pellet. The yeast cells are “fragile” at this point.
13. Add the following components into each tube on top of the cell pellet in this order (the order is important because the PEG “shields” the cells from the high concentration of lithium acetate):
 - 240 microliters of PEG (50% w/v)
 - 36 microliters of 1.0 M LiOAc
 - 5 microliters of salmon sperm DNA, 10 mg/ml (boil 5 minutes, then keep on ice)
 - 1-5 microliters (100 ng – 5 micrograms) of plasmid DNA
 - 65 microliters of sterile nano-pure dH₂O.
14. Vortex the cell pellet for at least 1 minute to resuspend the cells in the transformation mix.
15. Incubate the transformation mixes at 42°C for 15 minutes.
16. Centrifuge the cells at top speed in a microcentrifuge for 10 seconds. Remove the supernatant using a micropipet.
17. Gently resuspend the pellet in 200 microliters of sterile, nano-pure dH₂O by slowly and carefully pipetting up and down. (Be as gentle as possible at this step.)
18. Plate 100 microliters of the cell suspension onto a plate containing minimal medium that selects for the presence of the plasmid. Let the plates sit for 5-10 minutes to absorb the mixture before placing plates in the 30°C incubator. Colonies should be visible in 2-3 days.